LESSON 9:
APPLICATIONS OF SHOOT MERISTEM CULTURE

Virus-free Plants

Introduction

The objective of this Lesson is to study practical applications of shoot meristem culture. Its application mostly lies in production of disease- free plants.

Most of the horticultural and forest crops are infected by systemic diseases caused by fungi, viruses, bacteria, mycoplasma and nematodes. Pathogen attack does not always lead to the death of the plant but very often the infection caused by pathogens considerably reduces the yield and quality of crops. While pathogens are nearly always transferred in plants through vegetative propagation, viral diseases occur in virtually all seed propagated as well as vegetatively propagated crop species (Kartha 1984). Eradication of pathogens is highly desirable to optimise the yields and also to facilitate the movement of materials across the international boundaries. While plants infected with bacteria and fungi may respond to treatments with bactericidal and fungicidal compounds, there is no commercially available treatment to cure virus-infected plants. The therapeutic chemicals capable of eradicating virus from infected plants are not available though the use of virazole and vidarabine (anti metabolites) in the culture medium have resulted in the production of virus-free lily and apple plants (Pierik 1989).

To produce disease-free plants a healthy nucleus stock could be developed by selecting out one or more healthy plants and then multiplying them vegetatively, but where the entire population of a clone is infected the only way to obtain a pathogen-free plant is through tissue culture. Apical meristems in the infected plants are generally either free or carry a very low concentration of the viruses. However, the titre of the viruses increases in the older tissues corresponding to the increase in the distance from the meristern tips. Various reasons attributed to the escape of the meristems by virus invasion are:

a. viruses move readily in a plant body through the vascular system which in meristems is absent,

b. a high metabolite activity in the actively dividing meristematic cells does not allow virus replication and

c. a high endogenous auxin level in shoot apices may inhibit virus multiplication.

A knowledge of the gradient of virus distribution in shoot tips encouraged Holmes (1948) to obtain virus-free plants from infected individuals of Dahlia through shoot-tip cuttings. Morel and Martin (1952) applied tissue culture techniques for elimination of viral infection in plants. They cultured meristem tips excised from infected Dahlia and obtained disease-free plants. Since then the advances made in elimination of virus by tissue culture have been such that this approach has now become a popular horticultural practice.

The first application of meristem culture was to obtain virus-free plants of dahalias. In 1952, Morel and Martin isolated 100 um long meristems and cultured them to obtain virus-free shoots. Since then the technique of meristem culture has been greatly refined and used for obtaining plants free from viruses, viroids, mycoplasma and even fungi and bacteria in a range of crops. In India, some valuable clones of potato, sugarcane etc. have been freed from virus infections through meristem culture. Care must be taken to remove the apical meristem with as little surrounding tissue as possible to minimise the chances of virus particles being present in the explant. This application of meristem culture is of great value, particularly in the maintenance of breeding materials and germplasm exchange which are invaluable for any breeding programme.

Considerable expertise is required to dissect out the shoot apical meristem with only one or two leaf primordia (100-500um in length). Care has to be taken to prevent desiccation, and contamination by the virus present in the surrounding tissue. Generally, growth regulators (usually, small amounts, of an auxin and a cytokinin) are added to the medium to support shoot growth from the cultured meristems. In general, the larger the meristem explant, the greater the chances of its survival and shoot development. But the risk of infection by the virus also increases with explant size (Table 1.). Therefore, a compromise has to be reached between these two opposing forces in deciding the explant size.

TABLE 1. The frequency of virus-contaminated plants of Cymbidium recovered by culturing shoot meristems of different lengths.

Length of Shoot Meristem	Virus contaminated Plants
(um)	(%)
600	100
270	87
120	52

It may be emphasized that many viruses have been shown to be present within the apical meristem, e.g., carnation mottle virus, chrysanthemum stunt virus etc. Further, all the plants derived from meristem culture are not necessarily virus-free; many of them may even contain latent bacterial and/or fungal infections. Therefore, it is essential that each tissue culture raised plant must be tested, using specific tests, for the presence of a given virus or pathogen before it is claimed that the plant is free from the concerned virus/pathogen. Further, freedom must be claimed only from the specific virus/pathogen for which it has been shown to be free.

Thermotherapy and Meristem Culture

Conventionally, viruses are eliminated by thermotherapy of whole plants in which plants are exposed to temperatures between 35-40°C for a few minutes to several weeks depending on the 'host-virus combination. Thermotherapy is based on the fact that most viruses are killed at temperatures much below those which kill their host plants. Thermotherapy is usually

effective against isometric and thread-like viruses and against mycoplasmas, but is ineffective against many other viruses. Only a small proportion of plants survive heat treatment, and often these plants become susceptible to other diseases possibly due to inactivation of their defense mechanisms.

Thermotherapy is often combined profitably with meristem culture to obtain virus-free plants. In general, shoot tips are excised from heat-treated plants, but cultured meristems may also be given thermotherapy; the former is preferable since larger explants can be safely taken from heat-treated plants (this increases the survival rate of explants).

Potato virus S (PVS) and potato virus X (PVX) are not eliminated either by thermotherapy or by meristem culture alone. But, both these viruses have been eliminated by culturing meristems from heat-treated plants. But in case of some viruses, e.g., potato spindle tuber virus, plants recovered from thermo-therapy plus meristem culture have to be subjected to a second cycle of heat-treatment coupled with shoot tip culture for effective virus elimination. The heat-treatment may either be continuous or a daily cycle of high and low temperatures may be applied; the latter is often preferable. The duration of treatment may vary from few to several days, e.g., 8-18 weeks for PVX, depending on the host-virus combination. This is perhaps the most successful approach of virus elimination.

Cryotherapy

More recently, prolonged exposure to a low temperature (5°C) followed by shoot tip culture has proved quite successful in virus elimination; this is often called cryotherapy. For example shoot-tip cultures from Chrysanthemum plants treated with 5°C for 4 months yielded 67% plants free from chrysanthemum stunt virus (CSV), and 22% plants free from chrysanthemum chlorotic mottle virus (CCMV). The frequency of virus-free plants increased to 73% in case of CSV and to 49% in case of CCMV after a 7.5 month exposure to 5°C. Cold treatment is preferable to heat-treatment as it is less injurious to the plants and often more effective in virus elimination.

Chemotherapy

Some chemicals, e.g., virazole (ribavirin), cycloheximide, actinomycin D etc., which interfere with virus multiplication may be added into the culture medium for curing the shoot-tips of viruses; this is known as chemotherapy. For example, virazole was shown to eradicate potato virus X (PVX) from shoots derived from tobacco protoplasts. Virazole is effective against a range of animal DNA and RNA viruses. It has also been used to eliminate ORSV (odontoglossum-ringspot virus) from Cymbidium.

Virus-Indexing

It may be emphasized that all the plants obtained through meristem culture, with or without chemo- or thermotherapy, are not virus-free. Therefore, such plants have to be tested for the presence of the concerned virus(es). This is called virus indexing.

1. The simplest method for virus-indexing is to score the plants for the presence of specific symptoms produced by the relevant virus. However, the development of visible symptoms may take considerable time.
2. Alternatively, the saps from test plants may be used to inoculate highly sensitive and healthy indicator plants. An indicator plant for a virus is that plant species or variety which is highly susceptible to the virus and readily develops the symptoms.
3. A highly sensitive and precise technique for virus-indexing employs antibodies specific to the concerned virus(es). This is known as serological test. ELISA (enzyme-linked immunosorbant assay) is perhaps the most convenient, rapid and efficient test system especially when a large number of samples are to be handled.
4. DNA and RNA probes can also be used for virus detection but they are time-taking and are not amenable to large scale application in a manner similar to ELISA.

Maintenance of Virus-Free Stocks

Plants freed from viruses are maintained in insect-free glass-houses or cages. This is necessary to prevent re-infection by the viruses since such plants are as susceptible to virus infection as the parent variety from which they were obtained. A large scale multiplication of such plants can be done in areas where and during seasons when the chances of infection by the virus are minimal. However, the best and cheapest means of maintaining virus-free plants is in in vitro cultures.

Conclusion

Shoot tip culture is a very efficient method to produce virus-free plants. Virus elimination generally improves the yield by 20-90% over infected controls. However, elimination of viruses may increase the susceptibility of plants to other more severe viruses, since the presence of one virus confers resistance to infection by other viruses (virus cross protection). In addition, virus-free plants so obtained may rapidly become infected by the same virus once they are taken to the field. As a result, virus-free plants have been developed and used in breeding programs and for germplasm exchange, but they themselves are of little commercial interest.

Questions

1. What type of explant should be taken to produce virus- free plants?
2. What do you understand by the following:
   a. Thermotherapy
   b. Cryotherapy
   c. Virus- indexing.

Note